Quantification of soluble fibre in feedstuffs for rabbits and evaluation of the interference between the determinations of soluble fibre and intestinal mucin

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A B S T R A C T

This work compared the quantification of soluble fibre in feeds using different chemical and *in vitro* approaches, and studied the potential interference between soluble fibre and mucin determinations. Six ingredients: sugar beet pulp (SBP), SBP pectins, insoluble SBP, wheat straw, sunflower hulls and lignocellulose, and seven rabbit diets, differing in soluble fibre content, were evaluated. In experiment 1, ingredients and diets were analyzed for total dietary fibre (TDF), insoluble dietary fibre (IDF), soluble dietary fibre (SDF), aNDFom (corrected for protein, aNDFom-cp) and 2-step pepsin/pancreatin *in vitro* DM indigestibility (corrected for ash and protein, *iv*DMi2). Soluble fibre was estimated by difference using three procedures: TDF–IDF (SDF_{IDF}), TDF–*iv*DMi2 (SDF_{ivDMi2}), and TDF–aNDFom-cp ($SDF_{aNDFom\text{-}cp}$). Soluble fibre determined directly (SDF) or by difference as SDF_{ivDMi2} were not different (109 g/kg DM, on average). However, when it was calculated as $SDF_{aNDFom\text{-}cp}$ the value was 40% higher (153 g/kg DM, $P<0.05$), whereas SDF_{IDF} (124 g/kg DM) did not differ from any of the other methods. The correlation between the four methods was high ($r \geq 0.96$; $P \leq 0.001$; $n=13$), but it decreased or even disappeared when SBP pectins and SBP were excluded and a lower and more narrow range of variation of soluble fibre was used. In experiment 2, the *iv*DMi2 using crucibles (reference method) were compared to those made using individual or collective ankom bags in order to simplify the determination of SDF_{ivDMi2}. The *iv*DMi2 was not different when using crucibles or individual or collective ankom bags. In experiment 3, the potential interference between soluble fibre and intestinal mucin determinations was studied using rabbit intestinal raw mucus, digesta and SBP pectins, lignocelluloses and a rabbit diet. An interference was observed between the determinations of soluble fibre and crude mucin, as contents of TDF and apparent crude mucin were high in SBP pectins (994 and 709 g/kg DM) and rabbit intestinal raw mucus (571 and 739 g/kg DM). After a pectinase treatment, the coefficient of apparent mucin recovery of SBP pectins was close to zero, whereas that of rabbit mucus was not modified. An

Abbreviations: aNDFom-cp, α-amylase neutral detergent fibre corrected for ash and protein; CP, crude protein; IDF, insoluble dietary fibre; *iv*DMi2, 2-step pepsin/pancreatin *in vitro* DM indigestibility corrected for ash and protein; *iv*DMi3, 3-step pepsin/pancreatin/viscozyme *in vitro* DM indigestibility corrected for ash and protein; SBP, sugar beet pulp; SDF, soluble dietary fibre determined directly; $SDF_{aNDFom\text{-}cp}$, soluble dietary fibre estimated as TDF–aNDFom-cp; SDF_{IDF}, soluble dietary fibre estimated as TDF–IDF; SDF_{ivDMi2}, soluble dietary fibre estimated as TDF–*iv*DMi2; SFF_{ivDMi3}, *in vitro* soluble and fermentable fibre estimated as TDF–ivDMi3; TDF, total dietary fibre.

estimation of the crude mucin carbohydrates retained in digesta TDF is proposed to correct TDF and soluble fibre digestibility. In conclusion, the values of soluble fibre depend on the methodology used. The contamination of crude mucin with soluble fibre is avoided using pectinase.

1. Introduction

The influence of insoluble fibre, usually quantified as NDF (Mertens, 2003) on rate of passage and caecal fermentation has been well established in the rabbit (Gidenne, 1994; García et al., 2002). Soluble fibre affects caecal fermentation (Falcao-e-Cunha et al., 2004; Gómez-Conde et al., 2009; Rodríguez-Romero et al., 2011) and gut barrier function (Gómez-Conde et al., 2007) leading to lower mortality rate in rabbits (Trocino et al., 2013). However, there is no agreement in the method to quantify soluble fibre and the potential interference of soluble fibre with other substances when determining its digestibility (Graham et al., 1986). Soluble dietary fibre can be quantified directly (SDF, Prosky et al., 1985) or by difference between total dietary fibre (TDF) and NDF (Van Soest et al., 1991). When these methods are used, inaccuracies are unavoidable because of problems such as partial degradation of carbohydrates, incomplete extraction and precipitation of soluble fibre with the addition of ethanol or interference with other fractions of the feed (Hall et al., 1997; Prosky, 1999; McCleary et al., 2010; Martínez-Vallespín et al., 2011). Moreover, these methodologies might not evaluate correctly the true proportion of insoluble and soluble fibre in the digestive tract of the rabbit, because they prioritize the elimination of starch (utilization of hot buffers to gelatinize, hydrolyse and depolymerise the starch) and therefore the temperatures and pH values used are not within physiological ranges (Marlett et al., 1989; Monro, 1993). The two step pepsin/pancreatin *in vitro* dry matter indigestibility (corrected for ash and protein, *iv*DMi2) can be used to measure the insoluble fibre content of ingredients under more physiological conditions. For example, the validated *in vitro* digestion proposed by Carabaño et al. (2008) to simulate small intestine digestion uses temperatures, pH and time similar than those existing in the rabbit gut. When the *in vitro* method is used the filtration step of the digested fractions is done using crucibles. However, it has been shown that the use of the ankom technology would simplify this technique as has been shown previously for NDF and ADF and other *in vitro* methodologies (Komarek et al., 1994; Fay et al., 2005).

The quantification of TDF and soluble fibre in ileal digesta or faeces might be affected by the contamination with endogenous substances (Wilfart et al., 2007), like mucin, an endogenous glycoprotein mostly constituted by carbohydrates (>700 g/kg, Mantle and Thakore, 1988) that covers the mucosa and resistant to digestion. Mucin is precipitated in ethanol, as occurs with soluble fibre, and it may result in a lower apparent ileal and faecal TDF and soluble fibre digestibility compared to the real one or even in negative digestibility values (Graham et al., 1986; Gidenne, 1992). Likewise, determination of crude mucin content in the digesta, by ethanol precipitation (Lien et al., 1997; Leterme et al., 1998; Libao-Mercado and de Lange, 2007; Piel et al., 2004) may be overestimated due to the lack of specificity as the residue may be contaminated with proteins and soluble fibre (Mañas and Saura-Calixto, 1993; Leterme et al., 1996).

The aim of this work was to confirm whether the quantification of soluble fibre using methods with different chemical and *in vitro* approaches renders similar results when using different fibrous ingredients and diets for rabbits. A second objective was to study the potential interference between soluble fibre and intestinal mucin determinations.

2. Materials and methods

2.1. Experiment 1

Seven rabbit diets and six ingredients were selected based on their soluble, insoluble and total dietary fibre and used to compare the accuracy of quantifying soluble and insoluble fibre content using different methodologies. The diets were based on sources of fibre currently used in rabbit feeds, whereas the ingredients were chosen to increase the range of variation of TDF. The ingredients used were wheat straw (Pagran, PITE S.A., Tordesillas, Spain), sunflower hulls (SOS Cuétara, Andújar, Spain), lignocellulose (Arbocel RC fine, Rettenmaier Ibérica S.L., Barcelona, Spain), sugar beet pulp (SBP, Fipec, Nordic Sugar, Copenhagen, Denmark), SBP pectins (Betapec RU 301, Herbstreith & Fox, Neuenbürg, Germany) and insoluble SBP. The latter ingredient was obtained by boiling SBP in a solution (13.5 L water with 0.4 kg sodium alkyl sulphate and 100 g EDTA per kg SBP) with a pH value of 7 (adjusted with NaOH) for 1 h. Afterwards, the mixture was filtered through nylon tissue (46 μm pore), washed with water overnight at room temperature to remove only the soluble constituents, dried at 70 °C and ground. Additionally, seven rabbit diets were used in the present study. Four of the diets contained 330 g aNDFom/kg DM and 161 g crude protein (CP)/kg DM. The control diet contained 360 g wheat starch, 154 g casein, with 180 g wheat straw and 180 g sunflower hulls per kg. A second diet was obtained by substituting 60 g of starch of the control diet by SBP pectins. Two more diets were obtained by substituting part of the fibrous sources (0.4) of the control diet by either SBP or by the insoluble SBP fibre, respectively. Another three diets contained 341 g aNDFom and 199 g CP/kg DM (Gómez-Conde et al., 2007) and were obtained by substituting part of the alfalfa hay by oat hulls and soybean protein concentrate or a mixture of SBP and apple pulp. Ingredients and diets were analyzed for TDF and insoluble dietary fibre (IDF), SDF, aNDFom corrected for CP (aNDFom-cp), *iv*DMi2 and 3-step pepsin/pancreatin/viscozyme *in vitro* DM indigestibility (*iv*DMi3 corrected for ash and CP).

In ingredients and diets a total of 16 determinations were conducted for *iv*DMi2 and *iv*DMi3, six determinations for TDF, IDF and aNDFom-cp, and two determinations for SDF. Soluble fibre was also estimated as TDF–IDF (SDF_{IDF}), as TDF–*iv*DMi2 (SDF_{ivDMi2}), and as TDF–aNDFom-cp ($SDF_{aNDFom\text{-}cp}$). The *iv*DMi3 was used to estimate the soluble and fermentable fibre as TDF–*iv*DMi3 (SFF_{ivDMi3}).

2.2. Experiment 2

A simplified procedure of the *iv*DMi2 and *iv*DMi3 methods used to estimate insoluble fibre was studied. The reference method of filtering in crucibles (Carabaño et al., 2008) was compared with the use of individual or collective ankom bags. Four diets (control, and SBP pectin, SBP and insoluble SBP containing diets) and six ingredients (lignocellulose, SBP pectin, SBP, insoluble SBP, sunflower hulls and wheat straw) were used. The determinations of *iv*DMi2 and *iv*DMi3 were conducted at four different times. At each time the crucibles (conducted in duplicate for each ingredient, diet and time) and the individual (conducted in duplicate for each ingredient, diet and time) and collective ankom bags (1 jar including two bags/ingredient and time) methods were conducted simultaneously.

2.3. Experiment 3

The potential interference between soluble fibre and crude mucin determinations (both are quantified by precipitation in ethanol) was studied to clarify whether it could affect the determination of soluble fibre and TDF digestibility. Raw mucus, free of digesta, obtained from jejunum of a rabbit affected of epizootic rabbit enteropathy (Licois et al., 2006) was chosen as a reference because the lack of a standard crude mucin from rabbit. Raw mucus was analyzed for TDF and compared to TDF content of SBP pectins treated or not with pectinase (with no correction for ash and protein). Apparent crude mucin was determined in raw mucus, ileal digesta and faeces of adult rabbit, and three ingredients free of crude mucin (SBP pectins, SBP and lignocellulose). After crude mucin analysis the residue obtained from each sample was treated enzymatically with a pectinase (Sigma P2401, St. Louis, USA) to eliminate potential contamination of mucin with soluble fibre. All determinations were performed in triplicate.

2.4. Chemical analysis

All samples were ground at 1 mm. The general analysis procedures of the AOAC (2000) were used to determine the concentrations of DM (method 934.01), ash (method 942.05) and CP (method 968.06. Dumas method), TDF (method 985.29), IDF (method 991.42) and SDF (method 993.19). Samples were filtered without any celite to facilitate N-Dumas determination. For TDF, IDF and SDF determinations 1 g sample were used and values were corrected for ash and CP content. In SBP pectins was also determined a modified TDF by adding before the final precipitation with ethanol 10 units of pectinase (overnight at pH 4.5 at 25 °C; Sigma P2401, St. Louis, USA). The NDF was determined in 0.5 g of sample according to the method exposed by Mertens et al. (2002) using the filter bag system ankom technology, and a thermo-stable amylase without any sodium sulphite added. Total aNDF content was corrected for CP and ash (aNDFom-cp) as indicated for IDF determination. The *iv*DMi2 and *iv*DMi3 were performed as indicated by Carabaño et al. (2008), in which the indigestible residue was also corrected for CP and ash. These determinations estimate the dietary insoluble fibre (*iv*DMi2) and the *in vitro* insoluble and no fermentable fibre (*iv*DMi3). The method was modified by using ankom filter bags rather than crucibles to facilitate sample filtering. Samples (0.5 g) were weighed in filter ankom bags and put on a *Daisy*^{II} *incubator jar* (3.5 L and 30 filter bags/jar). Once digested, bags were washed three times with distilled water (the first one for 30 min at 40 °C with agitation), followed with ethanol and acetone to avoid the adherence of any residue outside the bag. Finally, the bags were dried at 103 °C for 24 h. Two bags without any sample were used as blanks in each jar.

Crude mucin was determined using the ethanol precipitation method as indicated by Lien et al. (1997) and adapted by Romero et al. (2011) using 1 g sample. The crude mucin residue is mainly composed of raw mucus, contaminated by non-covalently bound proteins (Leterme et al., 1996). To remove the soluble fibre retained in the mucin residue 10 mL of phosphate buffer at 25 °C with 10 units of pectinase (Sigma P2401, St. Louis, USA. One unit liberates 1.0 μmol of galacturonic acid from polygalacturonic acid/min at pH 4.0 at 25 °C) were added and incubated overnight. Then 15 ml of absolute ethanol was added to precipitate again crude mucin that was recovered by centrifugation at 1400 × *g* at 4 °C for 10 min. The supernatant was eliminated and the residue was dried overnight at 60 °C.

2.5. Statistical analyses

In experiment 1, results for TDF, insoluble and soluble fibre are presented as average values with a pooled standard deviation of the determinations. The main effect of method, on insoluble and soluble fibre determinations, was analyzed by using a mixed model including the sample as a random effect and considering all ingredients and diets as replicates ($n = 13$). Mean values were compared using a test of Bonferroni. Linear regressions between all measurements of insoluble and soluble fibre were determined and the slopes tested if they were different from 1.0 using a *t*-test. In experiment 2, the results obtained for *iv*DMi2 and *iv*DMi3 (obtained with crucibles *vs.* single ankom bags, or crucibles *vs.* collective ankom bags) were analyzed with a mixed model that included as main effects the method (crucibles *vs.* ankom bags) and the feedstuff

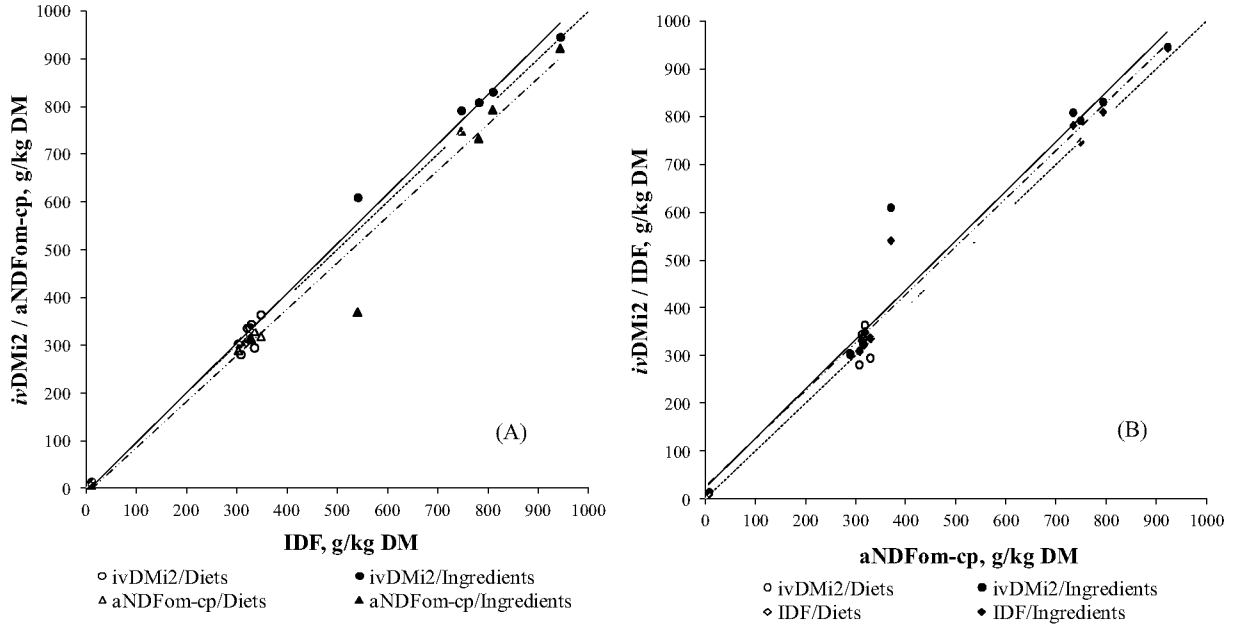

Fig. 1. Relationship between the different methodologies to quantify insoluble fibre using 7 rabbit diets and 6 ingredients ($n = 13$) [insoluble dietary fibre (IDF), amylase neutral detergent fibre (aNDFom-cp), and 2-step *in vitro* dry matter indigestibility (*iv*DMi2), all corrected for ash and crude protein]. All slopes were not different from 1.0 ($P \geq 0.21$).
(A) --- ○● *iv*DMi2 = −8.3 (±15.6) + 1.04 (±0.03) IDF, $r = 0.99$, rsd = 27.1, and – · · – · △▲aNDFom-cp = −12.2 (±26.7) + 0.97 (±0.05) IDF, $r = 0.98$, rsd = 46.6.
(B) --- ○● *iv*DMi2 = 22.9 (±38.8) + 1.04 (±0.07) aNDFom-cp, $r = 0.97$, rsd = 69.8, and — ◊⧫ IDF = 25.5 (±26.4) + 1.00 (±0.05) aNDFom-cp, $r = 0.98$, rsd = 47.4.

used. In this model, batch of analysis was considered a random effect. In experiment 3 results are presented as average values with their coefficient of variation. Statistical models were analyzed using InfoStat according to Di Rienzo et al. (2012).

3. Results

3.1. Experiment 1

The TDF content of diets and ingredients varied from 344 (control diet) to 959 (lignocellulose) g/kg DM (Table 1). Independently of the method used, determination of insoluble fibre was minimal for SBP pectins and maximal for lignocelluloses (10.4 and 937 g/kg DM on average, respectively), whereas the opposite results were observed for soluble fibre (924 *vs.* 16.4 g/kg DM, respectively; Table 1). Insoluble fibre values, considering each diet and ingredient as a replicate, determined with aNDFom-cp methodology was 8% lower than that measured with *iv*DMi2 (443 *vs.* 481 g/kg DM, $P < 0.05$), whereas the value obtained with IDF was intermediate and did not differ ($P > 0.05$) from aNDFom-cp and *iv*DMi2 (Table 2). Correlation between methods used to estimate insoluble fibre varied between 0.97 and 0.99 ($P < 0.001$; $n = 13$; Fig. 1), and all the slopes of regressions between the three different determinations of insoluble fibre were not different from 1.0. Moreover, the values of the 13 feedstuffs were as average lower for *iv*DMi3 than for aNDFom-cp (361 *vs.* 443 g/kg DM, $P < 0.001$; Table 1). The correlations between *iv*DMi3 of the 13 feedstuffs and insoluble fibre determinations (IDF, *iv*DMi2 and aNDFom-cp) were high ($r = 0.81$–0.88; $P \leq 0.001$).

The quantification of soluble fibre determined directly (SDF) and by difference as SDF_{ivDMi2} did not differ (109 g/kg DM, on average; $P > 0.05$; Table 2). However, the value was a 40% higher (153 g/kg DM; $P < 0.05$) when calculated as $SDF_{aNDFom\text{-}cp}$ than the average of these two procedures. The quantification of soluble fibre by difference as SDF_{IDF} did not differ ($P > 0.05$) from any of the other methods. The correlations between methods of soluble fibre estimation (SDF, SDF_{IDF}, SDF_{ivDMi2} and $SDF_{aNDFom\text{-}cp}$) of the 13 feedstuffs were also high ($r \geq 0.96$; $P \leq 0.001$; Fig. 2A). However, when SBP pectin was not included in the model the *r* values were reduced ($n = 12$; $r = 0.75$ and 0.83 for SDF with $SDF_{aNDFom\text{-}cp}$ and SDF_{ivDMi2}, respectively; $P \leq 0.005$; Fig. 2B) and even disappeared (between $SDF_{aNDFom\text{-}cp}$ and SDF_{ivDMi2}; figure not shown). When SBP was also excluded from the model (only feedstuffs with common soluble fibre content used), the correlation between methods remained high (0.85–0.93; Fig. 2C and D), with slopes not different from 1.0 (except between SDF and SDF_{IDF}; Fig. 2C).

3.2. Experiment 2

There were no differences for 2- and 3-step *iv*DMi when using crucibles or individual ankom bags, although differences among feedstuffs were significant ($P < 0.001$; Table 3). There were also no differences for *iv*DMi2 when using crucibles or

Table 1
Total (TDF), insoluble and soluble dietary fibre of experimental diets and ingredients using different methodologies (g/kg DM. Experiment 1).[a]

Item	Diets							Ingredients						Pooled standard deviation
	Control	Pectin	Sugar beet pulp	Insoluble SBP[b]	Oat hulls	Dehydrated alfalfa	Beet apple pulp	SBP pectin	Sugar beet pulp	Insoluble SBP	Ligno cellulose	Sunflower hulls	Wheat Straw	
TDF	344	398	400	354	414	409	435	934	646	805	959	841	785	7.96
Insoluble fibre														
IDF	327	322	349	330	336	309	304	10.7	541	782	944	810	747	5.05
ivDMi2	336	335	36.3	343	294	280	302	14.1	610	809	946	831	792	10.6
aNDFom-cp	314	315	318	312	329	307	289	6.4	369	733	922	793	748	8.75
*iv*DMi3[c]	306	295	259	241	295	252	207	7.8	167	265	907	748	744	11.1
Soluble dietary fibre														
SDF	14.5	23.4	24.9	8.9	54.1	66.8	96.0	922	106	23.2	1.9	32.0	−7.2	7.53
SDF_{IDF}[d]	16.9	74.9	51.3	23.2	78.2	99.6	131	924	106	23.1	14.4	30.4	38.4	9.84
SDF_{ivDMi2}[e]	7.8	62.8	36.3	11.2	120	129	133	920	36.3	−3.6	12.7	10.0	−6.8	13.7
$SDF_{aNDFom-cp}$[f]	30.3	82.0	82.3	42.2	85.4	102	146	928	278	72.7	36.7	48.0	37.2	12.9
SFF_{ivDMi3}[g]	38.1	103	141	113	119	157	228	926	479	540	52.2	93.1	41.1	14.4

[a] Number of determinations for each analysis: 16 for 2- and 3-step *in vitro* DM indigestibility (*iv*DMi2 and *iv*DMi3, respectively), six for TDF, six for insoluble dietary fibre (IDF), six for neutral detergent fibre (aNDFom-cp) and two for soluble dietary fibre (SDF). All these values were corrected for ash and protein.

[b] SBP: sugar beet pulp.

[c] Three-step *in vitro* insoluble and non-fermentable fibre.

[d] SDF_{IDF} = TDF − IDF.

[e] SDF_{ivDMi2} = TDF − *iv*DMi2.

[f] $SDF_{aNDFom-cp}$ = TDF − aNDFom-cp.

[g] SFF_{ivDMi3} = TDF − *iv*DMi3: *In vitro* soluble and fermentable fibre.

Table 2
Comparison of the different methodologies used to determine soluble and insoluble fibre considering each feedstuff (7 rabbit diets and 6 ingredients) as a replicate ($n = 13$).[a]

Item	Mean value, g/kg DM[b]	rsd	P-value
Insoluble fibre			
Insoluble dietary fibre (IDF)	470^{AB}	35.2	0.029
Two-step *in vitro* DM indigestibility (*iv*DMi2)	481^{B}		
Neutral detergent fibre (aNDFom-cp)	443^{A}		
Soluble fibre			
Soluble dietary fibre (SDF)	105^{b}	30.2	0.002
TDF[c]–IDF (SDF_{IDF})	124^{a,b}		
TDF–*iv*DMi2 (SDF_{ivDMi2})	113^{b}		
TDF–aNDFom-cp ($SDF_{aNDFom-cp}$)	152^{a}		

[a] Individual values of replicates are shown in Table 1.
[b] Mean values in the same column with a different superscript differ ($P < 0.05$).
[c] TDF: total dietary fibre.

collective ankom bags, but *iv*DMi3 was 1.5% higher when using collective bags than crucibles ($P < 0.001$), and differences among feedstuffs were also significant ($P < 0.001$). Correlations obtained for *iv*DMi2 and *iv*DMi3 using crucibles or ankom bags, either in single or collective digestions, were very high in all cases ($r \geq 0.997$; $P < 0.001$).

3.3. Experiment 3

The apparent crude mucin content in feedstuffs was important in SBP pectins and SBP diet (709 and 50.1 g/kg DM, respectively; Table 4). The apparent crude mucin content of raw mucus from rabbit jejunum was 739 g/kg DM, whereas

Table 3
Effect of the conventional method of filtering with crucibles and the use of ankom bags (individual, i-bag *vs.* collective bags, c-bag) in the determination of 2- and 3-step *in vitro* dry matter indigestibility (*iv*DMi2, *iv*DMi3, respectively) for rabbits (Experiment 2).[a]

	*iv*DMi2		*iv*DMi3		*iv*DMi2		*iv*DMi3	
	Crucible	i-bag	Crucible	i-bag	Crucible	c-bag	Crucible	c-bag
Diets								
Control	0.324	0.323	0.305	0.284	0.331	0.328	0.311	0.302
Pectin	0.327	0.331	0.282	0.273	0.322	0.330	0.293	0.280
Sugar beet pulp	0.361	0.352	0.264	0.273	0.357	0.380	0.263	0.273
Insoluble sugar beet pulp	0.331	0.321	0.242	0.241	0.335	0.322	0.246	0.243
Ingredients								
Lignocellulose	0.951	0.936	0.918	0.925	0.942	0.951	0.909	0.926
Sugar beet pulp pectins	0.012	0.019	0.0070	0.0010	0.017	0.014	0.0076	0.0012
Sugar beet pulp	0.603	0.604	0.166	0.191	0.600	0.598	0.165	0.188
Insoluble sugar beet pulp	0.802	0.784	0.267	0.266	0.803	0.780	0.246	0.243
Sunflower hulls[b]	–	–	–	–	0.822	0.845	0.742	0.769
Wheat straw[b]	–	–	–	–	0.783	0.786	0.739	0.759
Mean value of method	0.464	0.459	0.306	0.307	0.531	0.533	0.392	0.398
rsd	0.018		0.0095		0.013		0.0088	
P_{method}	NS[c]		NS		NS		<0.001	
$P_{feedstuff}$	<0.001		<0.001		<0.001		<0.001	

[a] Eight determinations for each analysis performed at four different times (in duplicated in each time), and at each time the reference method of filtering in crucibles and the method with individual and collective ankom bags (collective: 1 jar including two bags/feedstuff in each time) were performed.
[b] Values of sunflower hulls and wheat straw were excluded due to analytical problems with i-bag samples.
[c] NS: not significant ($P > 0.05$).

Table 4
Determination of apparent crude mucin content by ethanol precipitation method and its value once treated with pectinase (Experiment 3).[a]

Samples	Crude mucin (g/kg DM)	CV (%)	Crude mucin after pectinase treatment (g/kg DM)	CV (%)
Rabbit intestinal raw mucus[b]	739	7.11	726	6.94
Rabbit ileal content	86.4	14.9	74.6	13.3
Rabbit faeces	10.7	17.1	10.3	20.2
Sugar beet pulp pectins	709	9.94	1.77	19.5
Sugar beet pulp diet	50.1	11.3	0.16	18.5
Lignocellulose	5.52	16.6	–[c]	–

[a] In triplicate.
[b] Raw mucus from rabbit jejunum.
[c] Not determined due to the small amount of residue available.

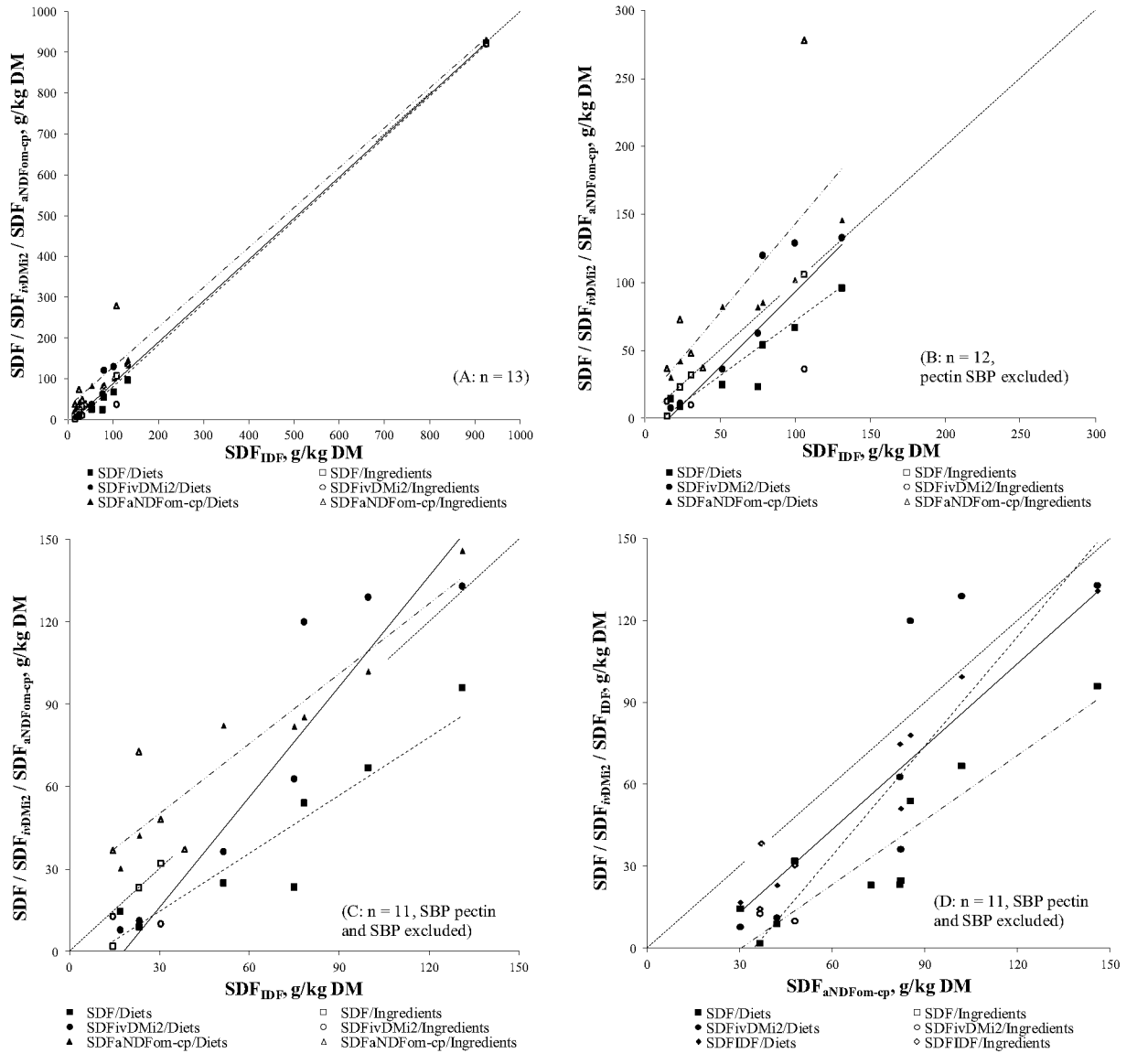

Fig. 2. Relationship between the different methodologies to quantify soluble fibre using 7 rabbit diets and 6 ingredients (A, $n = 13$), excluding sugar beet pulp (SBP) pectins (B, $n = 12$) or SBP pectins and SBP (C and D, $n = 11$) [soluble dietary fibre (SDF, direct analysis), or by difference as TDF–aNDFom-cp ($SDF_{aNDFom-cp}$), and TDF–*iv*DMi2 (SDF_{ivDMi2},), all corrected for ash and crude protein]. All slopes were not different from 1.0 ($P > 0.15$) unless indicated below.
(A) – · · · – △▲ $SDF_{aNDFom-cp} = 30.3\ (\pm 14.8) + 0.98\ (\pm 0.06)\ SDF_{IDF}$, $r = 0.98$, rsd = 47.2, ––– ○● $SDF_{ivDM2} = -12.4\ (\pm 9.3) + 1.01\ (\pm 0.04)\ SDF_{IDF}$, $r = 0.99$, rsd = 29.5, - - - □■ $SDF = -20.8\ (\pm 5.9) + 1.02\ (\pm 0.02)\ SDF_{IDF}$, $r = 0.99$, rsd = 18.8.
(B) – · · · – △▲ $SDF_{aNDFom-cp} = 11.7(\pm 24.7) + 1.31\ (\pm 0.36)\ SDF_{IDF}$, $r = 0.75$, rsd = 47.4, ––– ○● $SDF_{ivDMi2} = -17.9(\pm 16.1) + 1.11\ (\pm 0.23)\ SDF_{IDF}$, $r = 0.83$, rsd = 30.7, - - - □■ $SDF = -9.3\ (\pm 9.2) + 0.81\ (\pm 0.13)\ SDF_{IDF}$, $r = 0.88$, rsd = 17.7.
(C) – · · · – △▲ $SDF_{aNDFom-cp} = 24.5\ (\pm 7.3) + 0.85\ (\pm 0.11)\ SDF_{IDF}$, $r = 0.93$, rsd = 13.8, ––– ○● $SDF_{ivDMi2} = -24.3(\pm 11.5) + 1.34\ (\pm 0.18)\ SDF_{IDF}$, $r = 0.93$, rsd = 21.7, $P = 0.085$, - - - □■ $SDF = -6.4\ (\pm 7.9) + 0.70\ (\pm 0.12)\ SDF_{IDF}$, $r = 0.88$, rsd = 14.9, $P = 0.029$
(D) - - - ○● $SDF_{ivDMi2} = -46.1\ (\pm 21.7) + 1.33\ (\pm 0.28)\ SDF_{aNDFom-cp}$, $r = 0.85$, rsd = 31.0, – · · · – □■ $SDF = -23.9\ (\pm 9.4) + 0.79\ (\pm 0.12)\ SDF_{aNDFom-cp}$, $r = 0.91$, rsd = 13.4, $P = 0.094$, ––◊◆ $SDF_{IDF} = -17.4\ (\pm 10.5) + 1.01\ (\pm 0.14)\ SDF_{aNDFom-cp}$, $r = 0.93$, rsd = 15.1.

that of rabbit ileal digesta and faeces was 86.4 and 10.7 g/kg DM. Protein content of ileal and faecal mucins were 243 and 217 g/kg DM, respectively. When the apparent crude mucin residue, obtained after precipitation with ethanol, was treated with pectinase, soluble fibre was removed and the proportion of crude mucin recovered from SBP pectins and SBP were almost nihil (0.0025 and 0.0033, respectively), whereas that of rabbit raw mucus was not modified. The treatment of the apparent crude mucin residue with pectinase reduced the crude mucin content in ileal digesta by 14% (Table 4), whereas that of faeces decreased by 4%. The TDF content for rabbit intestinal raw mucus was positive and high (571 ± 36 g/kg DM), whereas TDF for SBP pectins was 994 ± 4.0 g/kg DM or 34.6 ± 8.1 g/kg DM when pectinase was used in TDF analysis (values no corrected for ash and protein).

4. Discussion

The quantification of soluble fibre depends on the method used to determine it, showing $SDF_{aNDFom\text{-}cp}$ the highest value. The differences observed might be accounted for the different analytical conditions used in extraction procedures (mainly temperature, but also pH and reagents) to remove starch and protein in each method (Marlett et al., 1989; Monro, 1993). This is especially important in pectin rich feedstuffs, like SBP, where the use of boiling NDF solution (containing EDTA, a chelating agent of calcium bound in pectin complexes) led to solubilization of a higher amount of substances than for *iv*DMi2 (278 *vs.* 36.3 g/kg DM, respectively; Fig. 2B), where the temperature was 40 °C. The use of SDF_{IDF} reported intermediate figures between $SDF_{aNDFom\text{-}cp}$ and SDF_{ivDMi2} but not different from each of them. This result might be explained by the use for SDF_{IDF} of the same temperature (100 °C for starch gelatinization) than for $SDF_{aNDFom\text{-}cp}$ but for a shorter time (20 *vs.* 60 min) and without EDTA. On the opposite, the temperatures used for SDF_{IDF} were higher than for SDF_{ivDMi2} (first 100 and after 60 °C *vs.* 40 °C, respectively). A temperature of 100 °C has been related to the β-elimination of polygalacturonic acids during extraction, especially in SBP (Albersheim et al., 1960; Knudsen, 1997). These differences in the quantification of soluble fibre were more clearly shown when a lower and more narrow and usual range of variation of soluble fibre content in feedstuffs for rabbits was used (excluding SBP pectins and SBP; Fig. 2C and D). The results of the current work do not allow to recommend any of the methods employed, because this decision does not only depend on the accuracy of chemical extraction but also on their correlation with the physiological traits.The use of the *in vitro* method where conditions were close to those observed *in vivo* would be more appropiated according to Monro (1993), as it would be expected to be better correlated to the physiological response, although this hypothesis must be confirmed.

In the current study, no or negligible differences in *in vitro* DM indigestibility were detected when crucibles or ankom bags (both in single or collective digestion) were used. In fact, the correlations obtained were higher (0.99) than those reported by Vogel et al. (1999) when studied the *in vitro* DM digestibility of forages for ruminants (0.92). These authors reported higher *in vitro* digestibility when using ankom bags than when using crucibles (0.602 *vs.* 0.563, respectively), whereas in the current study this difference was much lower. The differences in *iv*DMi3 between the use of ankom collective bags or crucibles might be accounted for their ability to be washed (Vogel et al., 1999; Adesogan, 2005).

In the present work, contents of apparent crude mucin and TDF in intestinal raw mucus and SBP pectins were high, because the use of ethanol in both methodologies led to precipitate both soluble fibre and intestinal mucins. This might affect the determination of the digestibility of soluble fibre and TDF, as suggested by Wilfart et al. (2007), and the quantification of crude mucin content. An alternative to improve the quantification of intestinal crude mucin content free of soluble fibre would be to remove this constituent from the residue using a pectinase. Our results showed that this method worked with rabbit jejunal raw mucus that resisted pectinase treatment, whereas this enzyme hydrolysed and solubilized soluble fibre. The treatment with pectinase of the crude mucin residue obtained from the ileal digesta allowed the elimination of the contamination with soluble fibre, according to the specificity of the enzyme used. This is a simple purification method for intestinal crude mucin that might be used to correct values of digestibility of TDF and soluble fibre, although the possibility of protein contamination remains (Devaraj et al., 1992; Leterme et al., 1996; Libao-Mercado and de Lange, 2007). For this reason, the correction should be done using only the mucin carbohydrate fraction that can be obtained discounting from crude mucin its protein content.

In contrast, the contamination with mucins of TDF and soluble fibre determined in the digesta is difficult to solve, as there is no commercial mucinase available to remove mucin specifically. The easier option to obtain a more accurate value of digestibility of soluble fibre and TDF might be to do a correction using the mucin carbohydrates content of digesta retained in TDF residue. The mucin carbohydrates retained in ileal or faecal TDF might be estimated as:

$$\text{Corrected digesta TDF (g/kg DM)} = \text{Digesta TDF (g/kg DM)} - \text{Mucus TDF (g/kg DM)},$$

where TDF from intestinal mucus (free of protein) is calculated as:

$$\text{Mucus TDF (g/kg DM)} = \text{Mucus proportion in digesta (g/kg DM)} \times 0.571,$$

where 0.571 is the proportion of TDF in raw intestinal mucus (see results of Experiment 3), and mucus proportion in digesta is calculated as:

$$\text{Mucus proportion in digesta (g/kg DM)} = \frac{\text{Crude mucin proportion in digesta (g/kg DM)}}{0.739},$$

where 0.739 is the crude mucin content of raw intestinal mucus (see Table 4). In addition the protein content of crude mucin must be discounted to estimate the mucin carbohydrate fraction.

According to these results further studies are warranted to evaluate precisely the potential impact of intestinal mucin on digestibility of TDF and soluble fibre in rabbits.

5. Conclusions

The estimation of soluble fibre contents of the feedstuffs tested depended on the method used. The election of the method will require additional studies to determine which one is better correlated with the animal response. The contamination of

crude mucin determination with soluble fibre in the ethanol precipitation method can be corrected by using a pectinase to remove soluble fibre. An estimation of the crude mucin carbohydrates retained in TDF is proposed to correct TDF and soluble fibre digestibility.

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